Amendments to the Specification

Please replace the paragraph at page 1, lines 4-7 with the following amended paragraph:

REALTED APPLICATIONS

This application is a continuation-in-part of U.S. Serial No. 09/461,580, filed December 15, 1999, now U.S. Patent No: 7,452,664, the entire teachings of which are hereby incorporated by reference in their entirety.

Please replace the paragraph at page 11, line 27 through page 12, line 4 with the following amended paragraph:

Figure 2C is an alignment of the evolutionarily conserved core domains of yeast (ySir2, ~~GenBank~~ GENBANK® Accession Nos: X01419, M21316, SEQ ID NO: 2; yHST1, ~~GenBank~~ GENBANK® Accession Nos: U39041, L47120, SEQ ID NO: 3); murine (mSir2α, SEQ ID NO: 4) and *Salmonella* (CobB, ~~GenBank~~ GENBANK® Accession No: U89687, SEQ ID NO: 5) Sir2 proteins. Identical amino acids are boxed. A putative NAD binding cleft is indicated by asterisks. Less conserved amino acids are shaded.

Please replace the paragraph at page 12, lines 12 through 14 with the following amended paragraph:

Figure 4A is a ~~Coomassie~~ COOMASSIE® blue stained gel of 6XHis-tagged recombinant yeast (r-y Sir2p) and murine (r-m Sir2α) Sir2 proteins purified with Ni-NTA agarose under native conditions. Arrowheads indicate each full-length protein.

Please replace the paragraph at page 13, lines 1 through 6 with the following amended paragraph:

Figure 5A depicts the amino acid sequences of the N-terminal tails of H3 (SEQ ID NO: 6) and H4 (mono AC, SEQ ID NO: 7, tetra Ac, SEQ ID NO: 8) peptides. The asterisks depict the site of acetylation (mono Ac, tetra Ac) to generate peptides with and without acetylated lysines. Human H3 ~~GenBank~~ GENBANK® Accession No: M26150, mouse H3 ~~GenBank~~ GENBANK® Accession Nos: M23459, 32460, 32461, 32462. Human H4 ~~GenBank~~ GENBANK® Accession Nos: M60749, M16707, mouse H4 ~~GenBank~~ GENBANK® Accession No: U62672.

Please replace the paragraph at page 14, lines 3 through 5 with the following amended paragraph:

Figure 8a is a ~~Coomassie~~ COOMASSIE® blue-stained SDS-PAGE gel of purified recombinant yeast (rSir2p) protein, mouse Sir2 (mSir2α) proteins and vector controls. Full length proteins are indicated by dots.

Please replace the paragraph at page 15, lines 16 through 18 with the following amended paragraph:

Figure 12b show the phylogenetic tree of yeast and mouse Sir2 core domains. The core domain sequences of three mouse homologs termed α, β, and and γ are compared on ~~Clustal~~CLUSTAL X and NJPLOT programs to generate the phylogenetic tree.

Please replace the paragraph at page 17, lines 16 through 23 with the following amended paragraph:

Figure 19 depicts the amino acid sequence alignment of the core domain of ySir2 (SEQ ID NO: 14), yHST1 (SEQ ID NO: 15), yHST2 ~~GenBank~~ GENBANK® Accession NO: U39063, (SEQ ID NO: 16), yHST3 ~~GenBank~~ GENBANK® Accession No: U39062, (SEQ ID NO: 17), yHST4 ~~GenBank~~ GENBANK® Accession No: NC_001136 (SEQ ID NO: 18), mSir2alpha (mSir2α, SEQ ID NO: 19), mSir2beta (mSir2β, SEQ ID NO: 20), mSirg (mSir2γ, SEQ ID NO: 21), and deduced amino acid sequences of Sir2-like core domains (~~GenBank~~ GENBANK® Accession No: A1465098, SEQ ID NO: 22; ~~GenBank~~ GENBANK® Accession No: A1465820, SEQ ID NO: 23; ~~GenBank~~ GENBANK® Accession No: A1466061, SEQ ID NO: 24).

Please replace the paragraph at page 25, line 24 thorough page 26, line 3 with the following amended paragraph:

The cell used in the methods of the ~~inventor~~ invention can be a yeast cell (e.g., *Saccharomyces cerevisiae*) or a mammalian cell, including somatic or embryonic cells, Chinese hamster ovary cells, HeLa cells, human 293 cells and monkey COS-7 cells. A collection of cells can form a tissue or an organism. The organism can be, for example, a vertebrate animal (e.g., a mammal, such as mice, rats, pigs dogs, cats, primates, and humans), nonvertebrate animal, such

as an insect (e.g., *Drospholia melanogaster*), nematode (e.g., *C. elegans*), or yeast (e.g., Saccharomyces cerevisioa). A "tissue" refers to a collection of similar cell types (such as epithelium, connective, muscle and nerve tissue).

Please replace the paragraph at page 36, line 28 through page 37, line 12 with the following amended paragraph:

The present invention also pertains to methods of increasing the life span or decreasing aging of a cell or an organism by administering to the cell or organism an agonist of Sir2 that increases the NAD-dependent deacetylation of histone proteins, a mono-ADP-ribosyltransferase (e.g., Sir2) or an agonist of a mono-ADP- ribosyltransferase of a nuclear protein. In a preferred embodiment, the nuclear protein is p53 or a histone protein, in particular H2A, H2B, H3 or H4. In a more preferred embodiment, the acetylation status [[if]]of lysine residues at positions 9 and/or 14 of H3 are altered and the lysine at position 16 of H4 is altered by altering the activity of Sir2. Preferably the mono-ADP-ribosyltransferase is a Sir2 protein (e.g., Sir2α, Sir2β, Sir2γ). Specifically, the core domain of a Sir2 protein (Figure 2B, 6A, 14a, 19; SEQ ID NOS: 2, 3, 4, 5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24) alters the NAD-dependent acetylation status of histone proteins, the mono-ADP- ribosyltransferase or the agonist of NAD-dependent acetylation status of histone proteins and/or mono-ADP-ribosyltransferase activity of the core domain of Sir2.

Please replace the paragraph at page 43, lines 3 through 20 with the following amended paragraph:

The Sir2 protein and nucleic acid sequence include homologues, as defined herein. The homologous proteins and nucleic acid sequences can be determined using methods known to those of skill in the art. Initial homology searches can be performed at NCBI against the ~~GenBank~~ GENBANK® (release 87.0), EMBL (release 39.0), and ~~SwissProt~~ SwissProt™ (release 30.0) databases using the BLAST and PSI-BLAST network services. (See, for example, Altshul, *et al.*, *J. Mol. Biol.* 215: 403 (1990); Altschul *et al. Nucleic Acid Res.* 25: 3389-3402 (1997); Altschul, *Nucleic Acids Res.* 25:3389-3402 (1998), the teachings of which are incorporated herein by reference). Computer analysis of nucleotide sequences can be performed using the

MOTIFS and the FindPatterns subroutines of the Genetics Computing Group (GCG®, version 8.0) software. Protein and/or nucleotide comparisons can also be performed according to CLUSTAL algorithms (Higgins, *et al.*, Gene, 73: 237-244 (1988)). Homologous proteins and/or nucleic acid sequences to the Sir2 protein and/or nucleic acid sequences that encode the Sir2 protein are defined as those molecules with greater than 25% sequences identity, also referred to herein as similarity, (e.g., 30%, 40%, 50%, 60%, 70%, 80% or 90% homology). The percent identity between the yeast (*S. cerevisiae*) SIR2 sequence and the murine SIR2α and SIR2β sequence is 45.9% (FIG. 2D). These particular sequence similarities and identities were determined using the CLUSTAL algorithm.

Please replace the paragraph at page 47, lines 14 through 26 with the following amended paragraph:

Antibodies can be raised to the Sir2 protein, Sir2-like protein, analogs, and portions thereof, using techniques known to those of skill in the art. (See, for example, Kohler *et al.*, *Nature 256*:495-497 (1975); Cole *et al.*, "Monoclonal Antibodies and Cancer Therapy", Alan R. Liss, Inc., pp. 77-96 (1985); Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons, Inc. (1999)). A mammal, such as a murine, rat, hamster or rabbit, can be immunized with an immunogenic form of the protein (e.g., mSir2α, mSirβ, or a peptide comprising an antigenic fragment of the protein which is capable of eliciting an antibody response). Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers, such as BSA or keyhole limpet hemocyanin, or other techniques, such as the use of Freud's adjuvant or ~~Titermax~~ Titermax™, well known in the art. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA, RIA or Western blots can be used with the Sir2 protein to assess the levels of antibody.

Please replace the paragraph at page 67, lines 13 through page 71, line 8 with the following amended paragraphs:

Northern blotting

The multiple tissue Northern blot (heart, brain, spleen, lung, liver, skeletal, muscle, kidney, testis) was purchased from ~~Clontech~~ Clontech™ (Palo Alto, CA). Prehybridization and

hybridization were performed at 65°C in 5X SSPE, 5X Denhardt's solution, 1% SDS and 0.1 mg/ml poly(A). The cDNA fragments of AA199012, AA105536, and AA260334 were used as probes for mSIR2α, β, and γ, respectively. The human β actin fragment from the manufacturer was used as a control probe. Between each blotting, the probe was stripped off by boiling the membrane in 0.5% SDS for 1 min.

Molecular cloning of mSIR2α

The mouse 15-day embryo 5'~~- STRETCH PLUS~~ STRETCH PLUS™ cDNA library (~~Clontech~~ Clontech™) was screened with the cDNA fragment of AA199012 as a probe. Five positive clones were obtained from approximately one million independent plaques. One of the five clones contained a 3.9kb cDNA fragment. The nucleotide sequence of this fragment was determined with an Applied Biosystems 374 automated sequencer. Although more 5' sequences of mSIR2α cDNA were obtained by 5' RACE with the mouse liver Marathon-Ready cDNA (~~Clontech~~ Clontech™), no stop codon could be found in the upstream of the first start codon (data not shown). This finding was confirmed on the genomic sequence of the mSIR2α gene (data not shown). Thus, the cDNA clone encodes the full-length mSir2α protein, as shown in FIG. 2B. The deduced amino acid sequence of mSir2α was aligned in the ~~Clustal~~CLUSTAL X and ~~SeqVu~~SEQVU 1.1 programs with other Sir2 family members.

Antibody production to mSIR2α

The 5' SalI-PvuI fragment of the mSIR2α cDNA was engineered to be cloned into BamHI site of pET16b vector (~~Novagen~~ NOVAGEN®, Madison, WI). The BL21 (DE3) pLysS bacterial strain that also has an extra copy of arginine tRNA gene was transformed with the resultant plasmid. A transformed bacterial clone was induced in 1mM IPTG at 37°C for 7hrs to produce 10xHis-tagged N-terminal fragment of the mSir2α protein. The mSir2α N-terminal protein was purified with Ni-NTA agarose (~~QIAGEN~~ QIAGEN®, Valencia, CA) under denaturing condition. Rabbit polyclonal antisera against this purified protein was produced at Covance Research Products (Denver, PA). Affinity purification of the antibody was performed with HiTrap NHS-activated column (~~Amersham Pharmacia Biotech~~ Amersham Pharmacia Biotech™, Piscataway, N.J.) conjugated with the dialyzed mSir2α N-terminal protein.

Western blotting and immunoprecipitation

Mouse NIH3T3 cells were lysed directly in Laemmli's sample buffer for Western blotting or in extraction buffer (20 mM Tris-HCl [pH7.6], 150 mM NaCl, 0.5% IGEPAL CA-630 (SIGMA, St. Louis, MO), 1 mM EDTA, 0.5 mM PMSF, 10μg/ml leupeptin, 10μg/ml pepstatin A, 10μg/ml aprotinin) for immunoprecipitation. The *in vitro* translated mSir2α protein was produced with the XbaI-linearized pBluescript containing the mSIR2α cDNA at SalI site and the STP3 T7 *in vitro* transcription/translation kit (~~Novagen~~ NOVAGEN®). For Western blotting, 17μg of the extract or 2μl of the *in vitro* translation mixture was run on a 4-15% gradient SDS-PAGE gel and transferred onto an Immobilon-P PVDF membrane (~~Millipore~~ MILLIPORE®, Bedford, MA). After preblocking strips of the membrane in TBS containing 0.1% Tween20 and 5% nonfat skim milk, the first antibody reaction was performed in the 1:2000 dilution of the affinity-purified antibody against mSir2α or control rabbit IgG. The mSir2α band was visualized with the secondary anti-rabbit IgG antibody conjugated with horse radish peroxidase and the ECL detection kit (Amersham Pharmacia Biotech). For immunoprecipitation, approximately 300μg of the extract was incubated at 4°C with 1μg of the affinity-purified mSir2α antibody for 1hr and then with Protein ~~A-Sepharose~~ A-SEPHAROSE® beads (~~SIGMA~~ SIGMA®) for another 1hr. The protein complex on beads was washed with extraction buffer three times and extracted in sample buffer. The protein was electrophoresed and blotted as described above.

Immunofluorescence

NIH3T3 cells or mouse embryonic fibroblasts were plated on 10-well multitest slideglasses (ICN Biomedicals, Aurora, OH). The cells were briefly washed with PBS, fixed in PBS containing 3.2% paraformaldehyde for 10 min, and then treated with PBS containing 0.5% IGEPAL-CA630 for 20 min. Preblocking reaction was performed with PBG buffer (PBS containing 0.2% cold water fish gelatin and 0.5% bovine serum albumin (~~SIGMA~~ SIGMA®)) at 37°C for 20 min. After washing cells in PBS-T (PBS containing 0.2% Tween20) briefly, the first antibody reaction was performed at 37°C for 1 hr in PBG containing the affinity-purified mSir2α antibody diluted to 1:500 and/or one of the anti-human nuclear antibodies (ANA-N for nucleolus or ANA-C for centromeres, ~~SIGMA~~ SIGMA®) diluted to 1:5 or 1:10 or control rabbit IgG. The cells were washed in PBS-T for 5 min twice. The secondary antibody reaction was performed at

37°C for 1 hr with the anti-rabbit IgG antibody conjugated with FITC (Jackson ImmunoResearch Laboratories, PA) and/or the anti-human IgG antibody conjugated with ~~Texas-Red~~ TEXAS-RED® (Vector Laboratories, CA). After washing in PBS-T for 5 min, the cells were counterstained in 200 ng/ml of DAPI for 1 min. They were washed in TBS-T for 5 min again and in dH_2O briefly, and then embedded under coverslips with ~~Vectorshield~~ VECTORSHIELD® (Vector Laboratories).

For the immuno-FISH of mSir2α and telomeres, the first antibody reaction for mSir2α was performed as described above. The cells with the first immune complex was then fixed with 3.2% paraformaldehyde for 10 min, permealized again in PBS containing 0.5% ~~IGEPAL-CA630~~ IGEPAL®-CA630 for 20 min, treated in 0.1M NaOH for 2 min to denature DNA, and immediately washed in ice-cold PBS. The synthesis of a telomeric probe, hybridization of the probe and visualization of the hybridized spots were done as described previously. The secondary antibody reaction to detect mSir2α was also done during the last visualization step.

All immunofluorescent digital images were obtained with the Nikon ~~Eclipse~~ ECLIPSE® TE300 fluorescent microscope equipped with a CCD digital camera (~~Hamamatsu~~ HAMAMATSU® Photonics, Japan) and the ~~Metamorph~~ METAMORPH® imaging system software (Universal Imaging Corp., PA).

Production of Recombinant Proteins

The yeast *SIR2* gene or the mSIR2a full-length cDNA was cloned into pET28a vector (~~Novagen~~ NOVAGEN®). BL21 (DE3) and BL21 (DE3) pLysS with an extra copy of arginine tRNA gene was transformed with the ySIR2 and mSIR2α plasmids, respectively. Each transformed bacterial clone was induced in 1 mM IPTG at 37°C for 1 hr. The induced 6XHis-tagged proteins were purified with Ni-NTA agarose under native condition (*see* FIG. 4A). The N-terminal fragment of mSir2α was prepared in the same way. The control elute was prepared from a bacterial clone carrying pET28a vector only. The recombinant proteins were aliquoted and kept at -70°C.

Site-directed mutagenesis of mSIR2α core domain was performed with the ~~GeneEditor~~ GeneEditor™ system (Promega, Madison, Wis.) according to the procedure provided by the manufacturer. The mutant recombinant proteins were prepared in the same way as described

above.

ADP-Ribosylation Assay

The typical reaction was performed in 50 or 100µl of the buffer containing 5 mM Tris-HCl [pH8.0 or 9.0], 4 mM $MgCl_2$, 0.2 mM DTT, 1µM cold β-NAD^+ (~~SIGMA~~ SIGMA®) and 8µCi nicotinamide adenine dinucleotide 5'-[α-^{32}P]triphosphate (NAD) as a donor of ADP-ribose (~1000 Ci/mmol, Amersham Pharmacia Biotech). To check the sensitivity to poly- or mono-ADP-ribosylation inhibitors, 3-aminobenzamide (ICN Pharmaceuticals, CA), benzamide, novobiocin and coumermycin A1 (~~SIGMA~~ SIGMA®) were added to the reactions prior to adding Sir2 proteins, respectively. The wild type or mutant recombinant proteins (0.5-1µg for ySir2p and 10 µg of mSir2α) were added with 4 µg of each histone purchased from Roche Molecular Biochemicals (Indianapolis, IN). For the peptide experiments, 5-10 µg of unacetylated or acetylated N-terminal tail peptides (amino acids 1-20) of H3 and H4 purchased from ~~Upstate Biotechnology~~ Upstate Biotechnology™ (Lake Placid, N.Y.) were used as substrates of the yeast and mouse Sir2 recombinant proteins.

Please replace the paragraph at page 71, lines 17 through 23 with the following amended paragraphs:

To analyze histone modification, 25 µl of 100% trichloroacetic acid (TCA) solution was mixed to the reaction mixture and left on ice for 15 min. The protein precipitates were centrifuged at 4°C and washed with 5 or 20% TCA solution twice. The pellets were dissolved in 15 µl of Leammli's sample buffer and boiled for 1.5 min. The proteins were electrophoresed in a 10-20% gradient SDS-PAGE gel. The gel was stained with ~~Coomassie~~ COOMASSIE® Brilliant Blue R-250 (GibcoBRL Life ~~Technologies~~ Technologies™, Frederick, MD) to check equal loading of histones, dried, and exposed to ~~Kodak~~ KODAK® X-OMAT film.

Please replace the paragraphs at page 72, line 1 through page 74, line 14 with the following amended paragraphs:

Transient Transfection Assay

Effector plasmids of mSIR2α were constructed using DNA fragments corresponding to amino acids 220-500 of the wild type and mutant mSIR2α amplified by PCR with ~~PfuTurbo~~ PfuTurbo® DNA polymerase (~~Stratagene~~ STRATAGEN®, La Jolla, CA) with primers that create EcoRI sites at both ends of each fragment. Fragments were cloned into the EcoRI site of pM mammalian expression vector (~~Clontech~~ Clontech™) to produce the N-terminal fusion protein to the GAL4 DNA binding domain. The luciferase plasmid, $pUAS_4tkluc$, which has four GAL4 binding sites in the upstream of the luciferase gene, was employed as a reporter gene. Plasmids preparations were made using the ~~QIAfilter~~ QIAfilter™ plasmid midi kit (~~QIAGEN~~ QIAGEN®).

NIH3T3 cells (10^6 cells) were plated a day before transfection. Cells were transfected using ~~SuperFect~~ SuperFect™ transfection reagent (~~QIAGEN~~ QIAGEN®) with 4 μg of the GAL4 DBD-core domain effector plasmid, 1μg of the luciferase reporter gene and 1.5 μg of SV40 promoter-driven β-galactosidase gene to normalize the transfection efficiency. The extracts of the transfectants were prepared 48 hrs after transfection. The luciferase assay was performed using the luciferase assay system kit (~~Promega~~ Promega™) and the Optocomp I luminometer (GM ~~Instruments~~ Instruments™, CT), according to the procedures provided by the manufacturers.

Production of Recombinant Proteins

The yeast *SIR2* gene or the mSIR2α full-length cDNA were cloned into pET28a vector (~~Novagen~~NOVAGEN®, WI). BL21 (DE3) and BL21(DE3)pLysS with an extra copy of arginine tRNA gene was transformed with the *SIR2* and *mSIR2α* plasmids, respectively. Each transformed bacterial clone was induced in 1 mM IPTG at 37°C for 1 hr. The induced 6XHis-tagged proteins were purified with Ni-NTA agarose under native conditions. The control elute was prepared from a bacterial clone carrying pET28a vector only. The recombinant proteins were aliquoted and kept at -70°C.

Deacetylation and ADP-Ribosylation Assays

The typical reaction of Sir2 deacetylase activity was performed in 50 µl of buffer containing 50 mM Tris-HCl [pH 9.0], 4 mM $MgCl_2$, 0.2 mM DTT, variable concentration of cold nicotinamide adenine dinucleotide (NAD) or NAD derivatives (~~SIGMA~~ SIGMA®, MO), 5-10 µg of the purified recombinant Sir2 proteins, and 10 µg of the histone H3 N-terminal tail peptide (amino acid 1-20) di-acetylated at positions 9 and 14 (~~Upstate Biotechnology~~ Upstate Biotechnology™, NY). This starting peptide material contains a contaminant with 100 Da smaller molecular weight, which also showed exactly the same patterns of deacetylation (data not shown). To detect the ADP-ribosylation activity, 8 µCi of NAD 5'-[α-^{32}P]triphosphate (~1000 Ci/mmol, ~~Amersham Pharmacia Biotech~~ Amersham Pharmacia Biotech™, NJ) was added to the same reaction containing 1 µ.M cold NAD. Histone H3 protein (4 µg) (~~Roche Molecular Biochemicals~~ Roche Molecular Biochemicals ™, IN) were used for this assay. All reaction mixtures were incubated at room temperature for 1 hr. Trichostatin A and coumermycin A1 (SIGMA®) were prepared in dimethylsulfoxide (DMSO, ~~SIGMA~~ SIGMA®), and 5µl of solvent or inhibitor was added to the reactions prior to adding Sir2 proteins.

Analysis of Deacetylated or ADP-Ribosylated Products

After the incubation, the products were precipitated at -20°C overnight by adding 50µl of distilled water and 25 µl of 100% trichloroacetic acid (TCA) solution. For high pressure liquid chromatography (HPLC), the precipitates were reconstituted in 5% CH_3CN and 0.1% trifluoroacetic acid (TFA) and run in the gradient concentration between 0.05% TFA and 0.043% TFA plus 80% CH_3CN on Hewlett Packard Model 1100 HPLC system with 214TP52 column (VYDAC, CA). The chromatograms at the absorbance of 210 nm were digitally recorded and analyzed by Hewlett Packard ~~ChemStation~~ ChemStation™ system (version A.06.03[509]). Fractions of samples were collected every 1 min by ~~Gilson~~ Gilson™ Fraction Collector Model 203. Peptide sequencing was done by the Applied Biosystems ~~Procise~~ PROCISE® 494 HT protein sequencing system. PTH amino acid chromatograms were recorded and analyzed by the ABI Model 610A2.1 data integration/analysis system.

Electron-spray mass spectroscopy (also referred to herein as mass spectroscopy) was done on the ~~PE Sciex Model API365~~ PE Sciex Model API365™ system. Matrix assisted laser desorption/ionization (MALDI) mass spectroscopy can also be used. The electron-spray mass

spectroscopy data were analyzed by the ~~BioMultiView program~~ BioMultiView program™ (version 1.3.1). To detect the ADP-ribosylated intact H3 protein, the pellets were dissolved in 15µl of Leammli sample buffer, boiled for 1.5 min and electrophoresed in a 10-20% gradient SDS-PAGE gel. The gel was stained with Coomassie Brilliant Blue to check equal loading of histones, dried, and exposed to ~~Kodak~~ KODAK® X-OMAT film. To analyze the ADP-ribosylated H3 peptides, 10µl of each reaction mixture was spotted on a cellulose TLC plate (~~EM Science~~ EM Science™, NJ). The chromatography was performed for 9-10 hrs in a TLC chamber containing the 65:5:3:2:29 mixture of isobutyric acid, pyridine, acetic acid, butanol and water (Scheidtmann, K. H., et al., J. Virol. 44:116-133 (1982)). The plate was dried and exposed to ~~Kodak~~ KODAK® X-OMAT film. The peptide spots were checked by ninhydrin staining.

Please replace the paragraphs at page 75, lines 13 through 23 with the following amended paragraphs:

Generation of Core Domain Mutants

Site directed mutations were generated in the plasmid pRS305-SIR2* using the ~~Gene Editor~~ Gene Editor™ system (~~Promega~~ Promega™, Madison, WI) according to the procedure provided by the manufacturer. Sequences were verified by Sanger sequencing methods. The mutants were then subcloned into pRS305-SIR2 and pET28a.

ADP-Ribosylation and Deacetylation Assays

BL21 with *SIR2* or the *sir2* mutants subcloned into pET28a (~~Novagen~~ NOVAGEN®) were induced with 1 mM IPTG for 1 hr. The recombinant proteins were purified by Nickel-NTA column under native condition. Ribosylation and deacetylation assays were performed using 1µg of recombinant protein for the ribosylation assay and 5µg for the deacetylation assay.

Please replace the paragraph at page 78, lines 1 through 12 with the following amended paragraph:

Life span assays were performed at 20°C. Adult hermaphrodites were picked (4- 10 per plate) from each strain and allowed to undergo one full generation at 15°C or 20°C. From these plates, individual L4s or young adults were picked to plates at 20°C containing 400 µ/ml FUDR ***~~(LENNY: PLEASE DEFINE THIS)~~*** which blocks DNA synthesis and causes animals to lay

eggs that do not develop and eliminates the need to transfer animals throughout the life span assay (Apfeld, J., *et al.*, *Cell, 95*:199-210 (1998)). Animals were tapped every two-four days and were scored as dead when they did not move after repeated taps with a pick. A limited number of experiments were carried out on plates ~~without~~ without FUDR by transferring adult animals every one-two days to new plates and these revealed the same, long life spans of *sir*-2.1 transgenic animals. All statistical tests were done using JMP 4.0 software.